bio-protocol Preprint

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Plasmid/short hairpin RNA construction and virus infection

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An abbreviated version of this protocol was published in Science Advances in Feb 2020

Interfering MSN-NONO complex–activated CREB signaling serves as a therapeutic strategy for triple-negative breast cancer

DOI: 10.1126/sciadv.aaw9960

Detailed protocol

Lentivirus Packaging and Infection Protocol

Prepare plasmids: core plasmids, two packaging plasmids (pMD2.G and pSPAX2), and the plasmid concentration>100ng/uL can be used.
Prepare Reagents: opti-MEM, PEI transfection reagent, Polybrene.

Part I. Packaging
Day1:
Plate 293T cells in 6-well plates. Cells should be ~80% confluent next day.

Day2:
Transfection plasmid into 293T cells, prepare the following mastermix:
100ul opti-MEM;
0.6ug pMD2.G;
1.2ug pSPAX2;
**2ug specific lentivector;
Incubate each individual mix for 5 min. at room temperature.
Then 8ul PEI is added, mixed *gently* and incubate at room temperature for 30min.
After incubation, add mixture on the SIDE of one well. Place plate in incubator for 36-48h.

Part II: Infection
Day3:
**Day before infection: Plate cells targeted for infection in 6-well plate. Cells should be ~30% confluent next day.

Day4:
Harvest virus particles by collecting medium, centrifuge at 1500 rpm for 5 min, and filter with 0.22um filter membrane.
Add 1ml media, then 0.5 ml virus, to 6-well plate.
Add 1.5 ul of 8 mg/ml polybrene (want final concentration of 8 ug/ml) to each well.
**Virus can now be stored at 4°C for a few weeks. For long-term storage, aliquot and store in -80°C.

Day5:
Change media.

Day6:
Add selection marker/puromycin (2 ug/ml) to each well containing virus, as well as to one of the control wells. **Cells in the control wells treated with antibiotics should display 100% death.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. qin, y. and Liu, S. (2022). Plasmid/short hairpin RNA construction and virus infection. Bio-protocol Preprint. bio-protocol.org/prep1663.
2. Qin, Y., Chen, W., Jiang, G., Zhou, L., Yang, X., Li, H., He, X., Wang, H., Zhou, Y., Huang, S. and Liu, S.(2020). Interfering MSN-NONO complex–activated CREB signaling serves as a therapeutic strategy for triple-negative breast cancer. Science Advances 6(8). DOI: 10.1126/sciadv.aaw9960

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